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See more with C-MORE: Addressing the need of robust cardiomyocyte morphological assessment

Rhys Wardman[1,2] and Joerg Heineke[1,2,*]
[1]Department of Cardiovascular Physiology, European Center for Angioscience, Medical Faculty Mannheim, Heidelberg University, 68167 Mannheim, Germany
[2]German Center for Cardiovascular Research (DZHK), partner site Heidelberg/Mannheim, Germany
*Correspondence: joerg.heineke@medma.uni-heidelberg.de
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Robust, comprehensive assessment of cardiomyocyte morphology is essential from research and clinical perspectives, but current methods predominantly rely on only limited parameters. Addressing this, Furkel et al. present, "C-MORE: A high content single cell morphology assay for cardiovascular medicine" in this issue of *Cell Reports Medicine*.

In this issue of *Cell Reports Medicine*, Furkel et al. introduce C-MORE, an open-source tool for single-cell morphology analysis in cardiomyocytes. C-MORE is presented as a ready-to-use workflow comprising a standardized experimental protocol including high-throughput image acquisition and analysis, which could be useful for basic research and translational approaches.[1] C-MORE addresses an urgent need of the cardiomyocyte research community.

Cardiomyocytes (accounting for up to 80% of heart mass) are delicately arranged both spatially and morphologically, facilitating normal heart rhythm and contractility.[2–4] In adulthood, cardiomyocytes display limited proliferative potential and thereby exhibit high degrees of morphological plasticity, determining changes in cardiac size, shape, and function and thereby playing a key role in the development of heart disease.

Hypertrophic heart remodeling constitutes the cardiac response to increased load or injury arising from physiological (e.g., exercise) or pathophysiological (e.g., hypertension or myocardial infarction) conditions and results primarily from increased cardiomyocyte mass and volume.[2,4,5] Demonstrating the importance of cardiomyocyte morphology, their growth patterns determine if concentric (primarily increased cardiomyocyte width resulting in thick ventricular walls and a small chamber) or eccentric (primarily increased cardiomyocyte length resulting in thin ventricular walls and enlarged chamber volume) hypertrophy ensues.[3–5] It is well known that cardiac pressure overload (e.g., due to aortic valve stenosis) results in concentric hypertrophy, while volume overload (e.g., due to mitral valve regurgitation, myocardial infarction, and ultimately end-stage heart failure) is associated with eccentric hypertrophy.[4,5] However, the key regulators determining concentric or eccentric cardiomyocyte growth remain poorly defined.

The ability to characterize the morphological profile of cardiomyocytes is therefore important from clinical and research perspectives. Understanding the dynamic changes occurring with physiological/pathophysiological remodeling could enable identification of novel therapeutic targets and monitoring of disease progression and therapeutic responses from biopsy samples in the clinic, where heart failure remains a serious problem.

Current *in vitro* cardiomyocyte models remain challenging for comprehensive morphological assessment. The main models are primary isolated cells (i.e., neonatal rat cardiomyocytes; NRCMs) and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). NRCMs are isolated from neonatal rat hearts by enzymatic digestion followed by cardiomyocyte enrichment. Caveats include phenotypic changes during isolation and impurity due to contamination with other cardiac cell types (e.g., fibroblasts and endothelial cells), contributing to phenotypic heterogeneity impeding robust characterization.[1]

The use of iPSCs has grown recently, allowing human cells with unique genetic backgrounds to be differentiated into numerous cell types otherwise difficult to obtain (i.e., cardiomyocytes). iPSC-CMs, generated by mimicking developmental cues driving cardiomyocyte differentiation, can be produced directly from patient samples allowing unique insights into genotype-phenotype relationships.[6] iPSC-CMs have been utilized as a model for numerous cardiac pathologies, offering invaluable insights into the molecular basis of cardiomyocyte function/dysfunction.[7] Furthermore, iPSC-CMs allow screening of therapeutic compounds based on a specific genetic background and screening novel drugs for efficacy over a range of genotypes and disease stages, offering exciting potential for development of personalized medicine and cell-based therapies.[6,7] However, variability within populations of iPSC-CMs, e.g., due to preparation variation, genetic heterogeneity, and differing differentiation states, also hinders consistent morphological assessment.[7]

Clinical assessment of cardiomyocyte morphology is an important indicator of disease state and responsiveness to therapeutic interventions.[1] The assessment of cardiomyocyte morphology currently relies on manual analysis and average values of limited selected morphological features (such as cell volume, size, length,

etc.). Current methodologies lack the capability to comprehensively assess cardiomyocyte morphology in a sufficient number of cells, and often data output is highly variable and lacks comparability.[1]

Methods for high-throughput morphological characterization of cells are essential for drug screening, extrapolating huge amounts of data on the phenotypic characteristics of cells imaged by automated microscopy, and profiling effects of novel therapeutics and uncharacterized molecules.[8] However, these methods are challenging for models with high degrees of inter-cellular variability and with diverse growth patterns such as cardiomyocytes.

Building on the readily available open-source Cell Profiler software, which provides a platform to morphologically assess the phenotype of cells in a high-throughput manner, C-MORE incorporates a series of pre-processing and data analysis steps tailored for cardiomyocyte analysis (such as Q/C and cardiomyocyte filtering, distribution assessment, cell-cycle assignment, meta-feature assignment, and calculation).[1,9]

Incorporating a high number of morphological features provides the power to reliably detect even small effects in otherwise variable data. Capable of analyzing 110,000 cells (each for 1,338 morphological features) and incorporating customizable marker selection, C-MORE can address wide-ranging biological questions. For example, the authors incorporate a fluorescent NFAT construct, a transcription factor which translocates from the cytoplasm to the nucleus upon hypertrophy induction, as well as fluorescent nuclear and cytoskeletal stains to provide a comprehensive overview of cellular changes upon hypertrophic stimuli. As another example, with the help of C-MORE, differences in patient-derived iPSC-CMs harboring a mutation associated with hypertrophic cardiomyopathy (p.R943x in myosin binding protein C3) were examined. Despite no significant difference in cell size, C-MORE observed distinct phenotypic patterns in homozygous and heterozygous cells compared to wild-type cells.[1,10]

Demonstrating potential clinical application, NRCMs were incubated with plasma from patients suffering aortic valve stenosis (AS) and subsequently treated by trans-catheter aortic valve replacement (TAVR) and compared to healthy controls. C-MORE detected a range of phenotypic features altered in NRCMs incubated with AS plasma, detecting attenuation of the majority of pathological features after incubation with plasma 1 week post-TAVR. This demonstrates potential versatility in C-MORE to detect disease and monitor response to therapeutic intervention.

Together, C-MORE offers potential applications as both a research and a clinical tool. Its use with functional screens (such as gene knock-out/CRISPR-Cas libraries) can help unravel the underlying mechanisms of pathological changes in cardiomyocyte morphology. As demonstrated, C-MORE also has potential to detect features on a single-cell basis and observe phenotypic "subclusters" of cells within heterogeneous populations, offering the potential in combination with single-cell OMICs/RNA sequencing methods to correlate gene expression to morphology on a single-cell basis. When the C-MORE algorithm is transferred to tissue (instead of isolated cells), robust morphological assessment of cardiomyocytes from patient samples can be utilized to assess disease state, and screening assays could monitor and predict therapeutic outcomes.

Moving into the age of "high-throughput" screens and personalized medicine, cardiomyocyte research must keep up. Hindered by complexity and lack of homogeneous models, cardiomyocytes are challenging to characterize and current methodologies are not adapted to this purpose. While the full accessibility and wide-ranging applications remain to be seen, the open-source C-MORE package addresses the need for a cardiomyocyte-centric approach.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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